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EXAMINER	
HUYNH, PHUONG N	
ART UNIT	**PAPER NUMBER**
1644	

DATE MAILED: 01/12/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/973,199	RANI ET AL.	
	Examiner	Art Unit	
	Phuong Huynh	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *Three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 October 2004*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2 and 5-15* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-2, and 5-15* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-2, and 5-15 are pending.

2. In view of the amendment filed 10/21/04, the following rejections remain.

3. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 103(a) that form the basis for the rejections under this section made in this Office Action:

> A person shall be entitled to a patent unless:
>
> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering Patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 1-2, and 11-15 stand rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No 5,753,228 (May 1998, PTO 892) or US Pat No 4,357,272 (Nov 1982, PTO 892) each in view of US Pat No 5,688,682 (Nov 1997; PTO 892) and Beasley *et al* (Food and Agricultural Immunology 12: 303-215, Sept 2000, PTO 892).

The '228 patent teaches a process for the production of egg yolk antibodies binding to any parasitic antigen wherein the reference method comprises the steps of selecting suitable poultry bird such as hens or chicken (See column 5, lines 6-9, in particular), immunizing the poultry birds such as the chicken with known complete adjuvant such as Freund's complete adjuvant containing heated killed and dried 1 mg/ml of M tuberculosis (See column 5, lines 13-21, Example 1, in particular). The '228 patent teaches that the adjuvant enhances the antibody responsiveness to the immunogen (See column 5, lines 13-18, in particular). The '228 patent teaches the bird such as leghorn hens, 21 weeks old are immunized with immunogen such as C

parvum in Freund's complete adjuvant and booster shots are given at intervals of five weeks (See column 8, lines 26-33, in particular). The reference method wherein the Eggs are collected, stored at 4 °C until processed. The '228 patent teaches that the advantages of antibodies from egg yolks of hyperimmunized hens are: it provides a continuous source of large quantities of uniform antibodies which can be easily collected and stored (column 1, lines 54-59, in particular) and whole chicken serum is also remarkably resistant to temperature and acidity (See column 5, line 49-51, in particular).

The '272 patent teaches a process for the production of egg yolk antibodies binding to any antigen wherein the reference method comprises the steps of selecting suitable poultry bird such as Leghorn chicken (See column 6, Example 1, Hens, in particular), immunizing the poultry birds such as the chicken with any antigen in the range of 1 to 5 mg/ml which is equivalent to 1000 to 5000 μg/ml intramuscularly in known incomplete adjuvant (See column 6, lines Immunization, lines 40-55, in particular). The '272 patent teaches the concentration of antigen used is not critical and varied from one antigen to another, but is generally in the range of 1 to 5 mg/ml. After the initial injection, the hens are immunized with additional injections (booster shot) at weekly intervals until the state of hyperimmunization is reached. The hyperimmunized eggs are collected and stores at 4 °C until use and this continuous over a period of 9 months (See column 6, lines 47-54, in particular). The '272 patent further teaches that if the antigen has low molecular weight (non-immunogenic), the immunogenicity of antigen can be enhance by cross-linking with carbodiimines (see column 5, lines 19-31, in particular). The '272 patent teaches that the antibody IgY titer produced ranges from 128 to 512 which is within the claimed range of 165-225 (see column 8, lines 6-10, in particular). The reference production of antibody is detectable 10 days after initial immunization and continued for 4 months (See column 7, lines 67 bridging column 8, lines 1-4, in particular). The '272 patent teaches the advantages of producing egg yolk antibodies are that it is comparatively easy to raise and keep chickens under conditions where they will produce antibody against the antigen desired and the antibody produced persists over such long periods such as the entire laying period (see column 4, lines 45-50, in particular).

The claimed invention in claim 1 differs from the teachings of the reference only that the process wherein the bird is immunized with 1000 μg conjugate 2,4,5 trichlorphenoxyacetic acid β-alanine mixed in 0.85 ml paraffin and 0.15 ml mannide monooleate in breast muscle, and the booster shots are given at a dose of 500 μg conjugate 2,4,5 trichlorphenoxyacetic acid β-alanine at the intervals of two, three five weeks as long as the bird lays eggs.

The claimed invention in claim 2 differs from the teachings of the references only that the process for the production of egg yolk antibodies wherein the desired hapten-protein conjugates having the binding properties of Hexachlorohexane.

The '682 patent teaches various adjuvant such as known complete adjuvant for vaccine Emulsigen, which is a paraffin oil in water emulsion that can be used in food animal and Freund's Incomplete adjuvant which is 15 percent (0.15 ml) by weight mannide monooleate and 85% (0.85 ml) paraffin oil (See column 4, lines 24-31, in particular). The reference adjuvants are useful for slowly releasing the vaccine into the animal and potentiating the immune response (See column 4, lines 31-32, in particular).

Beasley *et al* teach a method of making polyclonal antibody that binds to various pesticides such as Hexachlorohexane (HCH) by immunizing the rabbit with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) having a β-alanine spacer arm conjugated to KLH or Ova where the reference antibody having the binding property to Hexachlorohexane (HCH) (See page 207, Antibody production, in particular). Beasley et al further teach a method of making hapten conjugate to small molecule organo chlorine pesticide such as herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) having a β-alanine spacer arm by hydroxysuccinimide (See page 205, Materials and Methods, second full paragraph, page 206, structure Ib, in particular). The reference process of making conjugate hapten 2, 4,5-Trichloro phenoxy acetic acid (TCB) hapten binding to hexachloro hexane involves the steps of: (a) adding β-alanine spacer arm to 2.55 g of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in 5.95 ml thionyl chloride (50 mmol), (b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation; (c) stirring the product with β-alanine (9 mmol, 0.66g in 7.4 ml of 1M OH) at 0°C; (d) then warming the product over 16 hours at room temperature; (e) isolating the resulting acid by acidification; (f) partitioning the into ethyl acetate; (g) washing with water and brine and giving a yield of 0.5g or 16% of crude product hapten containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (See page 205, second full paragraph, in particular). Beasley et al teach 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is slightly more sensitive than other Hapten protein conjugate such as 1,2,4-TCB (See page 209, line 1, in particular) and 2,4,5 trichlorophoxyacetic acid (2,4,5-TCP) provides a relative simple targets for antibody development, enabling the detection of HCH residues by immunoassays after its conversion in samples to chlorobenzenes (See page 204, last paragraph, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the immunogen as taught by the '228 patent or the antigen as taught by the '272 patent for the immunogen such as 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) as taught by Beasley *et al* in adjuvant such as 15 percent (0.15 ml) by weight mannide monooleate and 85% (0.85 ml) paraffin oil as taught by the '682 patent for a process of making any egg yolk antibodies that bind to small molecule organo chlorine pesticide such as Hexachlorohexane as taught by the '228 patent, the 682 patent and Beasley *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because the '228 patent teaches that the advantages of antibodies from egg yolks of hyperimmunized hens because it provides a continuous source of large quantities of uniform antibodies which can be easily collected and stored (column 1, lines 54-59, in particular) and whole chicken serum is also remarkably resistant to temperature and acidity (See column 5, line 49-51, in particular). The '272 patent teaches the advantages of producing egg yolk antibodies are that it is comparatively easy to raise and keep chickens under conditions where they will produce antibody against the antigen desired and the antibody produced persists over such long periods such as the entire laying period (see column 4, lines 45-50, in particular). The Beasley et al teach 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) is useful for making antibody that binds to Hexachlorohexane (HCH) and 2,4,5-T is slightly more sensitive than other Hapten protein conjugate such as 1,2,4-TCB (See page 209, line 1, in particular). The '682 patent teaches that adjuvant is useful for slowly releasing the vaccine into the animal and in potentiating the immune response (See column 4, lines 31-32, in particular). The booster shots of immunizing the bird again and again at various intervals such as two, three or five weeks as long as the bird lay eggs is within the purview of one skill in the art at the time the invention was made because the '228 patent and the '272 patent teach booster shots maintain hyperimmunized antibody producing state and enhance the titer of the antibody. The recitation of collecting the eggs daily and stored at 40 °C until use is within the purview of one ordinary skill in the art at the time the invention was made because it is routine and customary to store away the collected egg at room temperature or refrigerated at 4°C until use. Claim 14 is included in this rejection because the sensitivity of the egg yolk antibody (polyclonal) is an inherent property of the antibody and would expect to be

equally sensitive to the polyclonal or monoclonal antibodies produced by mammals because they are immunized with the same immunogen.

Applicants' arguments in conjunction with the declaration of Bangalore Eshwar Anita Rani under 37 CFR § 1.132 filed 10/21/04 have been fully considered but are not found persuasive.

Applicants' position is that (1) the antibodies derived against the synthesis and identification of right conjugation to pesticides molecule involved obtain a correct epitope specific antibodies, which are able to recognize the pesticide of interest. The process of developing said antibodies involved the following requisites which have not been performed in any of the prior art and neither the present process has been suggested in the prior art such as the scheme shown on page 11-14, (2) the Trichlorobenzene hapten for the immunoassay of HCH synthesized by Beasley et al. was conjugated to OVA and keyhole limpet haemocyanin (KLH) and not to BSA for raising antibodies in rabbits. When trichlorobenzene containing a paminobuyric acid spacer arm that means increase of a methylene group was synthesized by one of the inventors (Scheme-5), and rabbits were immunized with conjugates of this hapten (HCH-2) we found that the antibodies recognized the hapten itself but not trichlorobenzene (the target molecule) in one of our trials. (A. Pasha and Amita rani (2003), unpublished work). Both HCH-I-HRP and HCH-2-HRP were employed as tracers but it did not help. (3) On the other hand, conjugating HCH-I hapten to BSA was most useful for raising antibodies in chicken. (4) The titer of antibody produced in chicken would not have been expected based on the teachings of the prior art. (5) Chicken antibody is better than the rabbit antibody in ELISA

In response to applicants' argument that the HCH synthesized by Beasley was conjugated to OVA and KLH and not BSA for raising antibodies in rabbits, the claimed method merely requires immunizing the bird with *any* hapten protein conjugate 2,4,5 trichlorophenozyacetic acid β-alanine. Further, it is within the purview of one ordinary skill in the antibody art to make nonimmunogenic small organic molecules such as HCH to be more immunogenic by chemical conjugation to a suitable carrier such as OVA and KLH as taught by Beasley et al. The claimed method does not require immunizing the specific BSA conjugated to HCH. This rejection would have been rejected under 35 U.S.C. 102(b) had Beasley et al teach immunizing chicken instead of rabbit using the reference conjugate. It is suggested that claims be amended to recite the specific site of conjugation, the specific carrier protein in the hepten protein conjugate and the binding

specificity of the yolk antibody produced by the claimed process, provided that the amended claims have support in the specification as filed.

In contrast to applicants' assertion that combination of reference does not suggest how or what type of antigen is needed for the pesticide antibodies, Beasley et al teach various antigens for making pesticide antibodies. Beasley *et al* teach a method of making polyclonal antibody that binds to various pesticides such as Hexachlorohexane (HCH) by immunizing rabbit with Hexachlorohexane (HCH) having β-alanine conjugated to ovalbumin or keyhole limpet hemocyanin or 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) β-alanine conjugated to ovalbumin or keyhole limpet hemocyanin where the reference antibody having the binding property to Hexachlorohexane (HCH) (See page 207, Antibody production, in particular). Beasley et al further teach a method of making hapten conjugate to small molecule organo chlorine pesticide such as herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) which conjugated to a β-alanine spacer arm by hydroxysuccinimide then conjugated to ovalbumin or keyhole limpet hemocyanin (See page 205, Materials and Methods, second full paragraph, page 206, structure Ib, in particular). Given that the method steps of making the antigen and the antigens are the same as that of the reference teachings, the IgY antibodies produced using the same antigen conjugate would obviously binds to the same organo chlorine pesticides. Applicants' attention is directed to the rejection mentioned above.

In response to applicants' argument that antigens used in the claimed method have dual attributes, i.e. non-toxic to rabbit or birds, and elicit good antibody response, the antigen conjugate taught by Beasley *et al* inherently is not-toxic to rabbit, otherwise the reference antigens would not have made good antibody response in rabbit if the rabbit can not sustain injection of non-cellular antigens for raising the antibodies. Further, the antigen conjugate taught by Beasley *et al* produces excellent antibody response in rabbit and merely good antibody response in chicken does not mean that the reference antigen conjugate completely fails to produce antibody response in chicken as taught by the combined references of record.

In response to applicants' argument that the antigens of the cited references have cellular origin (i.e. they are proteins), whereas the antigens of the present invention are non-cellular in origin, Beasley et al teach the antigens of non-cellular in origin (they are pesticides) (see entire document, page 205-207, in particular).

In response to applicants' argument that one cannot inject the pesticides directly into the birds as they are lethal, it is well known at the time the invention was made that birds have been used to produce IgY antibody to toxin (see '272 patent of record, in particular).

In contrast to applicants' assertion that the titer of antibody produced in chicken is at an average of 100mg/egg and estimated to be an output of 5g/hen which is almost 70 times more than the yield of the antibodies produced by rabbit and this result would not have been expected based on the teachings of the prior art, it is well known at the time the invention was made that egg yolk represents an alternative source of specific antibodies since each egg yolk can yield up to 200 mg of immunoglobulin as evidence by the teachings of the '228 patent teaches (of record, column 1, lines 54-59, in particular), Akita et al (of record, see page 629, col. 1, in particular).

In response to applicants' argument that chicken antibody is better than the rabbit antibody in ELISA, it is noted that the claims are drawn to a process of making chicken antibody, not chicken antibody.

In response to applicant's argument that the examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. In re McLaughlin, 170 USPQ 209 (CCPA 1971). The reason or motivation to modify the reference may often suggest what the inventor has done, but for a different purpose or to solve a different problem. It is not necessary that the prior art suggest the combination to achieve the same advantage or result discovered by applicant. See MPEP 2144. The strongest rationale for combining references is a recognition in the art that some advantage or expected beneficial result would have been produced by their combination. This recognition may be an expressed statement in a reference, an implication that can be drawn from one or more references or a convincing line or reasoning based upon established principles or legal precedent.

In response to the argument that interest in the commercialization of the present invention is evidence of non-obviousness, Applicants' arguments including the issue of commercialization of the present invention are not found persuasive of patentability when the claimed invention would flow logically from the teaching of the prior art of record. The combined teachings US Pat No 5,753,228 (May 1998, PTO 892) or US Pat No 4,357,272 (Nov 1982, PTO 892), US Pat No

5,688,682 (Nov 1997; PTO 892) and Beasley *et al* (Food and Agricultural Immunology 12: 303-215, Sept 2000, PTO 892) provide clear direction, motivation and expectation of success in making IgY antibodies that bind specifically to organ chlorine pesticide.

6. Claim 5 stands rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No 5,753,228 (May 1998, PTO 892) or US Pat No 4,357,272 (Nov 1982, PTO 892) each in view of US Pat No 5,688,682 (Nov 1997; PTO 892) and Beasley *et al* (Food and Agricultural Immunology 12: 303-215, Sept 2000; PTO 892) as applied to claims 1-2 and 11-15 mentioned above and further in view of McAdam et al (J Agric Food Chem 40: 1466-70, 1992; PTO 892).

The combined teachings of the '228 patent, the '272 patent, the '682 patent and Beasley *et al* have been discussed supra. Beasley et al further teach a method of making hapten conjugate to small molecule organo chlorine pesticide such as herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) which conjugated to a β-alanine spacer arm by hydroxysuccinimide (See page 205, Materials and Methods, second full paragraph, page 206, structure Ib, in particular). The reference process of making conjugate hapten 2, 4,5-Trichloro phenoxy acetic acid (TCB) hapten binding to hexachloro hexane involves the steps of: (a) adding β-alanine spacer arm to 2.55 g of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T).in 5.95 ml thionyl chloride (50 mmol), (b) refluxing for 1 hour and removing unreacted thionyl chloride by evaporation; (c) stirring the product with β-alanine (9 mmol, 0.66g in 7.4 ml of 1M OH) at 0°C; (d) then warming the product over 16 hours at room temperature; (e) isolating the resulting acid by acidification; (f) partitioning the into ethyl acetate; (g) washing with water and brine and giving a yield of 0.5g or 16% of crude product hapten containing 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (See page 205, second full paragraph, in particular). Beasley et al teach the impurity such as un-react hapten can be isolate by chromatography on silica gel (see page 205, last paragraph, in particular) using various solvents such as chloroform (see page 205, last paragraph) and methanol of choice (See page 211, first paragraph, in particular). The ratio of chloroform and methanol such as 85:15 as eluent for thin layer chromatography is within the purview of one skill in the art at the time the invention was made because it is a routine optimization to separate the product from the contaminant. The spraying with 2% o-tolidine in acetone for thin layer chromatography is within the purview of one skill in the art at the time the invention was made because it is a routine visualization in thin layer chromatography analysis. The Rf value of 0.45 and the melting rang of 169-70°C are inherent properties of the reference compound. Beasley et al further teach synthesizing NHS

ester of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T)-β-alaline using the same procedure as Triclopyr by dissolving in dichloromethane, adding N-hydroxysuccinimide and dicyclohexycarbodiimide as a coupling agent and stirring at room temperature and isolating the product using silica (See page 205, last paragraph, in particular). Beasley et al teach the most effective solvent for hexachlorocyclexane are methanol, acetone and hexane:acetone (4:1) (See page 210, Detection of Residues in Soil and Water, in particular).

The claimed invention in claim 5 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein the production of conjugate hapten 2,4,5-Trichloro phenoxy acetic acid β-alanine (TCB) hapten binding to Hexachloro hexane involves the steps of adding dimethylaminopyridine as a catalyst; stirring the mixture overnight and the temperature slowly raised to the room temperature; filtering and evaporing acetone and (r) separating the active ester as a colorless solid.

McAdam *et al* teach three approaches to hapten-protein conjugation such as coupling of β-alanine to organophosphate by dissolving β-alanine in dichloromethane, adding dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine as a catalyst, stirring the mixture at room temperature such as 20°C, filtering, drying and separating the active ester (See page 1467, column 1, first paragraph, in particular). McAdam *et al* further teach a method of coupling of activated ester such as Fenitrothion to hapten such as KLH, HRP or Ovalbumin (OA) using N-hydroxysuccinimide as a coupling agent for making the reference ogranophosphate Fenitrothion more immunogenic for antibody production (See page 1467, column 1, Coupling of Activated Feitrothion Succinimide Esters to carrier proteins, column 2, polyclonal and monoclonal antibody production, in particular). McAdam *et al* teach that hapten conjugates coupled through the spacer-arm such as alanine yield the most specific monoclonal and polyclonal antibody and higher affinity (See page abstract, page 1468 column 2, last paragraph, in particular). McAdam *et al* teach Monoclonal antibodies offer the advantage of potential scale up of production of any well defined antibody and polyclonal antibodies prepared the same way used in the same assay format is only slightly less sensitive (See page 1469, column 2, General Discussion, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to produce any egg yolk antibodies (polyclonal antibodies) binding to small molecule organochlorine pesticides as taught by the '228 patent, the '272 patent, the '682 patent and Beasley *et al* by synthesizing the active ester of any hapten-beta alanine by dissolving in dichloromethane, coupling to beta-alanine using N-hydroxysuccinimide and

dicyclohexylcarbodiimide as coupling agents as taught by Beasley *et al* and McAdam *et al* in the present of dimethylaminopyridine as a catalyst as taught by McAdam *et al.* From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because McAdam *et al* teach that hapten conjugates coupled through the spacer-arm such as alanine yield the most specific monoclonal and polyclonal antibody and higher affinity (See page abstract, page 1468 column 2, last paragraph, in particular). McAdam *et al* teach Monoclonal antibodies offer the advantage of potential scale up of production of any well defined antibody and polyclonal antibodies prepared the same way used in the same assay format is only slightly less sensitive (See page 1469, column 2, General Discussion, in particular). The recitation of adding dimethylsulphoxide (DMSO) drop wise to the mixture until the hapten dissolved is within the purview of one ordinary skill in the art at the time the invention was made because polar solvent such as DMSO dissolves similar polar compound (like dissolves like) since Beasley et al teach the most effective solvent for hexachlorocyclexane are methanol, acetone and hexane:acetone (4:1) (See page 210, Detection of Residues in Soil and Water, in particular). The recitation of melting range of 102-104 °C of 2,4,5-trichlorophenoxyacetic-beta alanine is inherent property of the compound 2,4,5-trichlorophenoxyacetic-beta alanine.

Applicants' arguments in conjunction with the declaration of Bangalore Eshwar Anita Rani under 37 CFR § 1.132 filed 10/21/04 have been fully considered but are not found persuasive. The Examiner's rebuttals stated above are incorporated here by reference.

7. Claims 6 and 8-9 stand rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No 5,753,228 (May 1998, PTO 892) or US Pat No 4,357,272 (Nov 1982, PTO 892) each in view of US Pat No 5,688,682 (Nov 1997; PTO 892) and Beasley *et al* (Food and Agricultural Immunology 12: 303-215, Sept 2000; PTO 892) as applied to claims 1-2 and 11-15 mentioned above and further in view of Deignan *et al* (Food and Agricultural Immunology 12: 77-85, March 2000; PTO 892) and Akita *et al* (J of Food Science 57(3): 629-634, 1992; PTO 892).

The combined teachings of the '228 patent, the '272 patent, the '682 patent and Beasley *et al* have been discussed supra.

The claimed invention in claim 6 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein harvesting of antibodies

as defined in step (g) of claim 1 (a) obtained egg yolk without rupturing the yolk; (b) adding 100 ml of Tris buffer for every 10 ml of yolk; (c) removing the precipitate by centrifugation; (d) adding to the supernatant the precipitate solution of magnesium chloride and phosphotungstic acid for centrifuging; (e) discarding the pellet; (f) adding to the supernatant a water solution protein fraction of 12% polyethylene glycol; (g) incubating for 10 minutes and then centrifuging again; (h) precipitating out the antibody; (i) adding 10 ml of 10mM phosphate buffer to dissolve the precipitate; (j) cooling the antibody solution to 0°C; (k) adding 10 ml of precooled ethanol; (l) centrifuging the solution at 4°C and dissolving the sediment in 10 mM phosphate buffer; and (m) dialyzing against phosphate buffer for 24 hour at 4°C to obtain the yield of antibodies.

The claimed invention in claim 8 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein the lipid from egg yolk is precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride and centrifuged obtaining the antibody yield up to 75% from supernatant.

The claimed invention in claim 9 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein the pH of the water soluble protein fraction obtained after the removal of the lipids is adjusted to pH 5.0 to further precipitate out the antibodies for obtaining a yield of 80-90%.

Deignan *et al* teach a comparative analysis of five published methods of purifying egg yolk immunoglobulin such as lipid removal by freeze and thaw at neutral pH of Jensenius et al (1981), precipitation with 3.5% polyethylene glycol (PEG) of Polson and von Wechmar (1980), precipitation with dextran sulphate and calcium chloride of Jensenius et al (1981), precipitation of with phosphotungstic acid and magnesium chloride of Vieria et al (1984) (See entire document, Lipid removal page 78 bridging page 79, in particular) and immunoglobulin precipitation by precipitation using 12% PEG of Polson & von Wedmar, 1980; Polson et al 1985) (See page 80, in particular). Deignan *et al* teach obtaining egg yolk without rupturing the yolk (page 78, egg yolk separation, in particular), follows by lipid removal using the method of Vieira et al by adding 100 ml of Tris buffer for every 10 ml of yolk; (c) removing the precipitate by centrifugation; (d) adding to the supernatant the precipitate solution of magnesium chloride and phosphotungstic acid for centrifuging; (e) discarding the pellet (See page 79, Precipitation with phosphotungstic acid and magnesium chloride, in particular), follows by immunoglobulin precipitation using the method of Polson & von Wedmar et al by adding to the supernatant a water solution protein fraction of 12% polyethylene glycol; (g) incubating for 10 minutes and then centrifuging again;

(h) precipitating out the antibody; (i) adding 5 ml of 10mM phosphate buffer to dissolve the precipitate; (j) cooling the antibody solution to 0°C; (k) adding 5 ml of precooled ethanol; (l) centrifuging the solution at 4°C and dissolving the sediment in 5ml of phosphate buffer; and (m) dialyzing against phosphate buffer for 24 hour at 4°C to obtain the yield of antibodies. Deignan *et al* teach that the advantage of removal of lipid from native egg yolk using a combination of polyanions and cations such as phosphotungstic acid and magnesium chloride it that it recovered the highest yield of 21.6 mg (range of 20.4-33.0) of protein per ml of egg yolk (See Figure 1, page 81, in particular) and the IgY purity as estimated by densitometry was 69.8%. Following lipid removal, immunoglobulin precipitation using 12% PEG gives the highest yield of 8.62 mg (range 8.39 to 8.83) or IgY per ml of egg yolk and this method was deemed the best (See page 82, Ig precipitation, Fig 2, page 82, Discussion, in particular).

Akita *et al* teach a process of purifying egg antibodies (IgY) by lowering the pH to 5.0 of the water soluble protein fraction (WSF) to further remove the lipid from said WSF and the highest yield of IgY such as 92.7 to 94.2 % is obtained between 5.0 to 5.2, respectively (See Table 1, page 631, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to harvest the egg yolk antibodies that bind to small molecule organo chlorine pesticides as taught by the '228 patent, the '272 patent, the '682 patent and Beasley *et al* using the solution of phosphotungstic acid and magnesium chloride follows by Ig precipitation using 12% PEG as taught by the Deignan *et al* and further remove the lipid from said WSF by lowering the pH to 5.0 as taught by Akita *et al*. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Deignan *et al* teach that the advantage of removal of lipid from native egg yolk using a combination of polyanions and cations such as phosphotungstic acid and magnesium chloride is that it recovered the highest yield of 21.6 mg (range of 20.4-33.0) of protein per ml of egg yolk (See Figure 1, page 81, in particular) and the IgY purity as estimated by densitometry was 69.8%. Following lipid removal, immunoglobulin precipitation using 12% PEG gives the highest yield of 8.62 mg (range 8.39 to 8.83) or IgY per ml of egg yolk and this method is deemed the best (See page 82, Ig precipitation, Fig 2, page 82, Discussion, in particular). The recitation of precipitated out lipid from egg yolk "twice" using the precipitating solution of phosphotungstic acid and

magnesium chloride is within the purview of one ordinary skill in the art at the time the invention was made because it is an obvious variation of the teaching of Deignan *et al* who teaches that the advantage of removal of lipid from native egg yolk using a combination of polyanions and cations such as phosphotungstic acid and magnesium chloride would give the IgY purity as estimated by densitometry was 69.8%, which is within the limit of "up to" 75%". Akita *et al* teach that the advantage of lowering the pH to 5.0 of the water soluble protein fraction (WSF) further removes the lipid from said WSF and the highest yield of IgY such as 92.7 to 94.2 % is obtained between 5.0 to 5.2, respectively (See Table 1, page 631, in particular).

Applicants' arguments in conjunction with the declaration of Bangalore Eshwar Anita Rani under 37 CFR § 1.132 filed 10/21/04 have been fully considered but are not found persuasive. The Examiner's rebuttals stated above are incorporated here by reference.

8. Claims 7 and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No 5,753,228 (May 1998, PTO 892) or US Pat No 4,357,272 (Nov 1982, PTO 892) each in view of US Pat No 5,688,682 (Nov 1997; PTO 892) and Beasley *et al* (Food and Agricultural Immunology 12: 303-215, Sept 2000; PTO 892) as applied to claims 1-2 and 11-15 mentioned above and further in view of Akita *et al* (J of Immunological Methods 160: 207-214, 1993; PTO 892) or Hatta *et al* (Agric Biol Chem 54(10): 2531-2535, 1990; PTO 892).

The combined teachings of the '228 patent, the '272 patent, the '682 patent and Beasley *et al* have been discussed supra.

The claimed invention in claim 7 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein the harvesting of antibodies can also be conducted as follows: (a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane; (b) adding for every 10 ml of yolk, 10 ml of distilled water; (c) adding about 0.15% of kappa-carrageenan and left to stir for 30 minutes at room temperature; (d) filtering and centrifuging the solution for 15 minutes; (e) passing through the DEAE-Sephacel column prepared with 20 mM phosphate buffer at pH 8.0; (f) eluting with 0.2 M phosphate buffer pH 8.0; (g) collecting the eluate and the absorbance read at 280 nm; and (h) pooling and storing the peak fractions containing the antibody at 4°C.

The claimed invention in claim 10 differs from the combined teachings of the references only that the process for the production of egg yolk antibodies wherein the yield of antibody is to the extent of 73%.

Akita *et al* teach a process of isolating egg yolk immunoglobulin such as obtaining egg yolk from the egg shell without rupturing the yolk membrane from immunized hens; adding about 0.15% (w/v) of carrageenan (120 mg in 80 ml distilled water, which equal to 0.15%) and mixing and stirring for 15 minutes at room temperature which is about 20° C, centrifuging it to separate the water-soluble protein faction from the yolk lipoproteins (see page 210, right column, Fig 4, in particular). Akita *et al* teach the reference method yield 89% or 7.3 mg/ml of egg yolk (See caption in Figure 4, in particular). Akita *et al* teach the IgY in water-soluble protein fraction is purified by gel filtration such as passing through Sephadex G-25 column with the appropriate buffer and collecting the eluate by monitoring the absorbance at 280 nm (See page 210, Gel filtration, in particular). Akita *et al* further teach the hyperimmunized eggs are collected and store at 4°C until use (See page 208, column 2, immunization, in particular).

Hatta *et al* teach a process of isolating egg yolk immunoglobulin, IgG, a livetin protein, using several natural gums such as carrageenan and xanthan gum) by (a) obtaining the egg yolk from the eggshell without rupturing the yolk membrane from immunized hens; (b) adding about 0.1% (w/v) of kappa carrageenan (60 mg of carrageenan in 40 ml of distilled water) to the egg yolk in order to separate the water-soluble protein faction from the yolk lipoproteins by centrifugation (See page 2534, Diagram 1, Table 1, in particular); filtering the water-soluble protein fraction through filter paper, and passing through the DEAE-Sephacel column prepared with 20 mM phosphate buffer at pH 8.0 (see page 2533, column 1, Purification of IgY from egg yolk, in particular); eluting the yolk antibodies with 0.2 M (200mM) phosphate buffer pH 8.0; collecting the peak fractions by monitoring at 280 nm (See page 2534, Diagram 1, Table 1, in particular). Hatta *et al* teach the purity of IgY obtained by the reference method was 98.3% with a yield of 73% (See page 2534, column 1, second full paragraph, in particular). Hatta *et al* teach natural gums such as kappa carrageenan are effective as precipitant of yolk lipoproteins and the gum has been used as a food ingredient, so that IgY prepared by this method should be suitable for oral administration (See page 2534, column 2, Discussion, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to harvest the egg yolk antibodies that bind to small molecule organo chlorine pesticides as taught by the '228 patent, the '272 patent the '682 patent and Beasley *et al* using the process of separating lipoprotein by carrageenan and gel filtration such as DEAE-Sephacel column chromatography as taught by Akita *et al* and Hatta *et al.* From the combined

teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

One having ordinary skill in the art would have been motivated to do this because Akita et al teach that purification of IgY from egg yolk by carrageenan has no adverse effect on the immunoreactivities of IgY and the yield in terms of purity is 89% (See page 210, caption in Fig 4, abstract, in particular). Hatta *et al* teach natural gums such as kappa carrageenan are effective as precipitant of yolk lipoproteins and the gum has been used as a food ingredient, so that IgY prepared by this method should be suitable for oral administration (See page 2534, column 2, Discussion, in particular).

Applicants' arguments in conjunction with the declaration of Bangalore Eshwar Anita Rani under 37 CFR § 1.132 filed 10/21/04 have been fully considered but are not found persuasive. The Examiner's rebuttals stated above are incorporated here by reference.

9. No claim is allowed.

10. **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a).

Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). A shortened statutory period for response to this final action is set to expire THREE MONTHS from the date of this action. In the event a first response is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the statutory period for response expire later than SIX MONTHS from the date of this final action.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Phuong Huynh "NEON" whose telephone number is (571) 272-0846. The examiner can normally be reached Monday through Friday from 9:00 am to 5:30 p.m. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (571) 272-0841. The IFW official Fax number is (571) 273-8300.

12. Any information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

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